

Optimum Microencapsulation Conditions of Lemongrass Essential Oil Microcapsule Using Complex Coacervation Method

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Abstract

Microencapsulation of essential oil provides new novelty applications in textile industries where the technology allows the addition of insect-repellent, thermoregulated, antibacterial or fragrance to all types of textile substrate. In this study, lemongrass essential oil (LEO) was microencapsulated using a complex coacervation technique. The effects of LEO formulations on particle diameter and encapsulation efficiency was investigated using Response Surface Methodology (RSM). Chitosan (CH) and Gum Arabic (GA) were used as wall materials, and the LEO microcapsules formulation was developed with 17 different formulations, using Design Expert 13.0 software. Each formulation parameters and its interaction significantly affect the response ($p < 0.05$). The mean particle diameter size of the produced LEO microcapsules was found ranging from 6 μm to 12 μm . and the encapsulation efficiency of LEO microcapsules varied between 78.59 ± 0.01 to 85.32 ± 0.56 %. The suggested optimal formulation condition by the predicted model was at 30g of LEO mass, 2g of CH mass, and 1g of GA mass with desirability of 0.982. This formulation are able to achieve a particle diameter of 12 μm and an encapsulation efficiency of $85.21 \pm 0.06\%$.

1. Introduction

Lemongrass (*Cymbopogon flexuosus*), belonging to the grass family Poaceae, is a perennial crop renowned for its diverse industrial applications. The name "lemongrass" derives from its lemony aroma characteristic [1]. Lemongrass is mostly grown for its essential oil (EO) due to its therapeutic properties for anticancer, analgesic, and antibacterial [2]–[4]. The EO produced from lemongrass has several components that are well-known for their antibacterial, anticancer, and allelopathic qualities, including citral (a combination of neral and geranial), limonene, geraniol, and geranyl acetate. These compounds are used in the formulation of environmentally friendly pesticides [5].

Lemongrass essential oil (LEO) is widely used in the food industry [6], [7], as well as in the perfume and textile industries [8]–[10]. In textiles, LEO is used for insecticidal and fragranced textiles, where finishes are applied to the fabrics to impart functional properties. However, the industrial utilization of LEO is limited by inherent properties such as hydrophobicity, volatility, and susceptibility to degradation [7]. The utilization of microcapsule technology has emerged as an effective solution to address this issue. Microencapsulation technology shields plant EOs from the external environment, thereby significantly enhancing their stability and prolonging their storage life. This technological advancement ensures the preservation of the EO purity and provides a robust foundation for their further development and utilization in various applications [11].

Numerous studies have investigated the production of LEO microcapsules by a variety of techniques, including spray drying and coacervation. In order to determine their impact on the diameter of the encapsulated material, several of these research have investigated the use of biodegradable polymers as wall materials, such as chitosan (CH), gum Arabic (GA), maltodextrin from maize and cassava, and cyclodextrins, either individually or in combination [12]–[16]. The use of these biodegradable polymers has drawn a lot of attention during the past two decades because of their high mucoadhesive, film-forming ability, good release properties, and lack of toxicity [17].

Recently, numerous researchers have published on the microcapsules made of CH and GA [14], [18]–[21]. GA is widely recognized as one of the most utilized polysaccharides in various industries due to its active surface properties and exhibits low viscosity [14]. GA has a negatively charged surface due to the presence of carboxyl groups (–COOH) in it. This characteristic enables GA to form complexes with proteins or other polysaccharides possessing positive charges through a process known as coacervation [22]. CH on the other hand, is a natural polysaccharide compound obtained through the deacetylation of chitin [17]. CH is characterized by a multitude of amino (–NH₂) and hydroxyl (–OH) groups, resulting in surfaces that can be ionized to carry a positive charge. Microcapsules derived from CH exhibit the capability to modulate drug release, consequently enhancing the bioavailability of drugs, particularly those that are water-insoluble [23].

The study by Zhang *et al.* highlights the advantage of combining GA and CH in encapsulation processes. In their evaluation of lavender oil encapsulation using various materials including GA, sodium caseinate, gelatine, CH, β -cyclodextrin, and polyvinyl alcohol, it was found that the use of pure GA resulted in an encapsulation efficiency of approximately 20%, however, the combination of GA and CH yielded significantly higher encapsulation efficiency of 31%. Furthermore, the GA-CH microcapsules exhibited improvements in loading capacity and release rate compared to those formed with pure GA [11]. It was reported that the yield for microcapsules made with pure GA was higher, and this could possibly be due to the higher viscosity of CH. In the research by Wijesirigunawardana and Perera, lime oil was encapsulated using CH and GA as wall materials. The lime oil microencapsulated cotton fabric demonstrated notable antibacterial activity upon mechanical crushing and after a mild washing [18]. Similarly, Sharkawy *et al.* observed sustained antibacterial activity from limonene and vanillin microcapsules produced using GA and CH grafted onto cotton fabric. This shows that the combination of these two polymers as wall material is significant [20].

This study aimed to produce LEO microcapsules by complex coacervation method using CH and GA as wall materials, as well as to investigate the effects of the different formulation conditions on the particle diameter and encapsulation efficiency. The best desirable process conditions for LEO microencapsulation were determined using Response Surface Methodology (RSM). To the best of our knowledge, no previous work reported the formulation of LEO with chitosan/gum Arabic microcapsules. Determining the best formulation of the LEO microcapsules could contribute to the quality of the encapsulated material.

2. Materials and Methods

Microcapsules were formed using core material and shell material. In the present study, LEO was selected as the core material, and it is 100 % pure and certified organic. It was obtained from Hiqili (Guangzhou) Supply Chain Technology Co. Ltd. Shell material was used to encapsulate the core material according to its compatibility with the core material and functionality. CH and GA were selected as shell materials. Tween 20 and sodium tripolyphosphate were used as emulsifiers and crosslinking agents, respectively. All the chemicals and reagents were used as received without further purification. The process was conducted using the procedure outlined by Sharkawy *et al.* [19]. Modifications are made as necessary for the specific application requirements.

2.1 Formation of Microcapsules

This study used a complex coacervation method to produce LEO microcapsule, as shown in Figure 1. First, 1% (w/v) chitosan solution was prepared by dissolving chitosan in 0.1 N acetic acid and left under magnetic stirring overnight. This process needs to be done to ensure complete dissolution of the materials. Subsequently, 1% (w/v) gum Arabic solution was obtained by dissolving GA in distilled water with continuous magnetic stirring at 45°C for 2 hours. Next, CH and GA solutions were mixed and added to the LEO along with 2.58 ml of Tween 20 as the emulsifier. The specific quantities of LEO, CH, and GA used in this study are detailed in Table 1. The experimental

design layout was formulated using Box-Behnken design in Design Expert software (Version 13.0, State Ease, Inc., Minneapolis, USA).

In the emulsification process shown in Figure 1, the mixture was emulsified at a speed of 8000 rpm for 10 minutes using a homogenizer. Then, the microcapsule solution was continuously stirred for 30 minutes at 450 rpm. The temperature was gradually decreased from 40°C to 5°C with the aid of an ice bath. The final step involved hardening the microcapsules by adding 2 ml of a 10% (w/v) sodium tripolyphosphate solution and stirring at 400 rpm for 3 hours. The sodium tripolyphosphate to CH ratio in all formulations was maintained at 1:2 (wt%). The resulting microcapsules were then stored in suspension for further analysis.

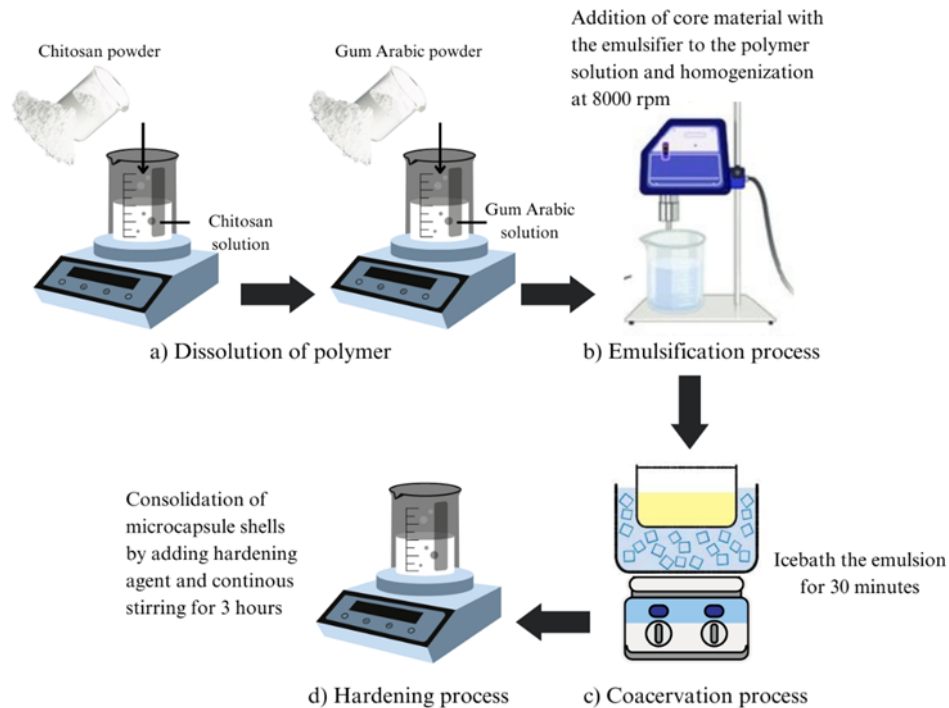


Fig. 1 The process for LEO microencapsulation

2.2 Microcapsules Characterization

The particle size of LEO microcapsules was observed using an advanced optical microscope at 10x and 20x magnification. The microcapsule solutions were mounted on a clean glass slide and covered by a cover slip. 150 particles were randomly selected, and the diameter was recorded before calculating the mean particle diameter. Each formulation was measured in triplicate. The encapsulation efficiency was calculated as the ratio of LEO mass (g) in the microcapsules and the LEO mass (g) added to the microencapsulation mixture, according to Equation (1).

$$\text{Encapsulation efficiency (\%)} = \frac{\text{mass (total)} - \text{mass (non-encapsulated)}}{\text{mass (total)}} \times 100 \quad (1)$$

2.3 Response Surface Optimization Experiment

Response Surface Methodology (RSM) was employed to obtain the optimal encapsulation conditions for LEO microcapsules. The influence of three independent variables; LEO mass (A), CH mass (B), and GA mass (C), on the response variables, 1. mean particle diameter, and 2. encapsulation efficiency, was evaluated using the Box-Behnken design (BBD). Each variable range in this study was selected based on preliminary studies and previous literature. This ensures that the chosen ranges were both realistically achievable and legitimate from a scientific standpoint. The levels (e.g., low, medium, high) were set to cover the entire range of each variable comprehensively. This allows for the detection of both linear and non-linear effects, as well as interactions between variables. The results were analyzed using Design Expert software (Version 13.0, State Ease, Inc., Minneapolis, USA). Table 1 represents the experimental design layout for the optimization process generated by the software, while Table 2 shows the factors and their corresponding levels. A total of 17 experimental runs, including centre points, were conducted randomly as per the specified run order. The statistical significance of

the model was determined by a p-value below 0.05. For the validation, one point was selected from the sample space of the data, and the response was predicted using the model. Then, the predicted experiment was analyzed and conducted. The percentage of errors was calculated using the formula below.

$$\text{Percentage of error (\%)} = \left[\frac{\text{Experimental value} - \text{predicted value}}{\text{predicted value}} \right] \times 100 \quad (2)$$

Table 1 Independent variables and their levels for the Box-Behnken design (BBD) used in the response surface methodology (RSM)

Factor/Levels	A: LEO mass (g)	B: CH mass (g)	C: GA mass (g)
-1	5.0	0.5	1.0
0	17.5	1.25	2.5
1	30.0	2.0	4.0

Table 2 Independent variables and their levels for the Box-Behnken design (BBD) used in the response surface methodology (RSM)

Run No.	A: LEO mass (g)	B: CH mass (g)	C: GA mass (g)
1	30.0	2.0	2.5
2	17.5	0.5	4.0
3	17.5	1.25	2.5
4	17.5	1.25	2.5
5	17.5	1.25	2.5
6	30.0	1.25	4.0
7	5.0	1.25	4
8	17.5	1.25	2.5
9	17.5	1.25	2.5
10	5.0	0.5	2.5
11	5.0	2.0	2.5
12	17.5	2.0	1.0
13	30.0	1.25	1.0
14	30.0	0.5	2.5
15	5.0	1.25	1.0
16	17.5	2.0	4.0
17	17.5	0.5	1.0

2.4 Statistical Analysis

The results were expressed as means \pm standard deviation (SD) to show variations in the various experiments. The Design Expert 13.0 software was used to determine the optimum formulation of LEO microcapsules. Values expressed are means of the three replicate determinations. An analysis of variance (ANOVA) test was completed to evaluate the significance of the effects ($p < 0.05$) [24].

3. Results and Discussion

3.1 Model Fitting

Table 3 shows the obtained outcome for mean particles and encapsulation efficiency of the microcapsules from the 17 experimental runs as suggested by Design Expert software. It is observed that mean particle diameter size ranging from 6 μm to 12 μm . The encapsulation efficiency of LEO microcapsules varied from 78.59 ± 0.01 to 85.32 ± 0.56 % as shown in Table 3. The lowest encapsulation efficiency was obtained from run 11 at LEO mass of

5g, CH mass of 2g, and GA mass of 2.5 g whereas the highest encapsulation efficiency was obtained from run 1 at LEO mass of 30 g, CH mass of 2 g and GA mass of 2.5 g.

These results were further analyzed using variance analysis to investigate the contributing impacts of the individual factors considered and their interactions. The effectiveness of the RSM model is best explained through the satisfaction of model performance by measuring the determination coefficients (R^2), F-values, lack of fit, coefficient of variation, adjusted R^2 , and p values (the value less than 0.05 indicates significant and vice versa). Table 4 and Table 5 present the results obtained for the ANOVA optimization for mean particle diameter and encapsulation efficiency. It can be observed that the two models of responses (mean particle diameter and encapsulation efficiency) are significant at 0.0106 and 0.0054. In Table 4, factor C and interaction AB are significant with p-value <0.05, while in Table 5, factor A is the only significant factor for encapsulation efficiency. Factor C is important for particle diameter in microcapsules due to its emulsifying properties, ability to increase solution viscosity, film-forming capabilities, and compatibility with other agents. This helps stabilize emulsions and form protective films, resulting in smaller and more uniform particle sizes, ensuring stability and controlled release of microencapsulated formulations. The interaction between factors A and B is significant due to their complementary properties, improving encapsulation efficiency, reducing particle size, enhancing stability, and providing synergistic antimicrobial effects. Lemongrass essential oil's significance for encapsulation efficiency is due to its chemical composition, need for protection and stabilization, and benefits from efficient encapsulation. This preserves its volatile components and enhances its functional properties, making the interaction between LEO and encapsulating agents like chitosan crucial for achieving high encapsulation efficiency and effective products.

The lack of fit for mean particle diameter and encapsulation efficiency were 0.3873 and 0.155, respectively, which is not significant ($p > 0.05$), implying the model could adequately fit the response data [27], [38]. Lack of fit was insignificant relative to the pure error [25]. A lack of-fit F-value this large could occur at a probability of 38.73% and 15.5% due to noise. Considering that we want the model to fit, a non-significant lack of fit is beneficial and desirable. The R^2 values for mean particle diameter and encapsulation efficiency are 0.8942 and 0.6107 respectively. This indicates that the predictive models can explain at least 89% of the results variations. The CV values for mean particle diameter and encapsulation efficiency are 11.38 and 1.43, respectively. According to earlier studies, a lower value of CV below 10% shows that the experimental results are reproducible and dependable experimental [24]. Hence, the encapsulation efficiency prediction models are a good fit.

Table 3 Design layout and experimental results for LEO microcapsules

Run No.	Microcapsules parameters			Responses	
	A: LEO mass (g)	B: CH mass (g)	C: GA mass (g)	Mean particle diameter (μm)	Encapsulation efficiency (%)
1	30.0	2.0	2.5	12	85.32 \pm 0.56
2	17.5	0.5	4.0	6	83.5 \pm 0.24
3	17.5	1.25	2.5	7	83.85 \pm 0.19
4	17.5	1.25	2.5	8	83.37 \pm 0.07
5	17.5	1.25	2.5	7	82.31 \pm 0.56
6	30.0	1.25	4.0	6	84.93 \pm 0.31
7	5.0	1.25	4	7	82.22 \pm 0.02
8	17.5	1.25	2.5	6	83.37 \pm 0.17
9	17.5	1.25	2.5	8	82.04 \pm 0.30
10	5.0	0.5	2.5	10	79.68 \pm 0.21
11	5.0	2.0	2.5	9	78.59 \pm 0.01
12	17.5	2.0	1.0	7	83.19 \pm 0.05
13	30.0	1.25	1.0	9	84.86 \pm 0.24
14	30.0	0.5	2.5	9	83.7 \pm 0.18
15	5.0	1.25	1.0	8	83.19 \pm 0.07
16	17.5	2.0	4.0	6	82.89 \pm 0.06
17	17.5	0.5	1.0	7	82.57 \pm 0.03

Table 4 Model summary statistics for mean particle diameter of LEO microcapsules

Source	Particle diameter (µm)				
	Sum of Squares	df	Mean Square	F-value	p-value
Model	46.92	9	5.21	6.58	0.0106*
A-LEO	1.12	1	1.12	1.42	0.2724
B-Chitosan	1.13	1	1.13	1.42	0.2724
C-Gum Arabic	4.5	1	4.5	5.68	0.0487
AB	6.25	1	6.25	7.88	0.0262*
AC	1	1	1	1.26	0.298
BC	0	1	0	0	1
Residual	5.55	7	0.7929		
Lack of Fit	2.75	3	0.9167	1.31	0.3873**
Pure Error	2.8	4	0.7		
Cor Total	52.47	16			
Std. Dev.	0.8904				
CV (%)	11.38				
R ²	0.8942				
Adjusted R ²	0.7582				
Predicted R ²	0.0781				

*Significant

**Not Significant

Table 5 Model summary statistics for encapsulation efficiency of LEO microcapsules

Source	Encapsulation Efficiency (%)				
	Sum of Squares	df	Mean Square	F-value	p-value
Model	28.66	3	9.55	6.8	0.0054*
A-LEO	28.61	1	28.61	20.36	0.0006
B-Chitosan	0.0364	1	0.0364	0.0259	0.8745
C-Gum Arabic	0.0091	1	0.0091	0.0065	0.937
AB	1.836	1	1.836	1.649	0.239
AC	0.2704	1	0.2704	0.242	0.637
BC	0.378	1	0.3782	0.339	0.578
Residual	18.27	13	1.41		
Lack of Fit	15.88	9	1.76	2.95	0.155**
Pure Error	2.39	4	0.5983		
Cor Total	46.93	16			
Std. Dev.	1.19				
CV (%)	1.43				
R ²	0.6107				
Adjusted R ²	0.5209				
Predicted R ²	0.2348				

*Significant

**Not Significant

3.2 Effects of Independent Variables and Interactions on the Response Variables

Mean particle diameter and encapsulation efficiency were analysed in three-dimensional (3D) plots to explore the interaction effect of independent variables on the optimisation of LEO microcapsules condition. The combined effect between two factors and mean particle diameter is shown in Figure 2. Figure 2(a) shows a response surface plot for the interaction effect of LEO and CH to a fixed 2.5g of GA on particle diameter. From the figure, it can be

observed that using 30 g of LEO and 2 g of CH was exhibited for about 12 μm of particle diameter which produced a bigger size of microcapsules compared to 5g of LEO with 0.5g of CH which produced about 10 μm to 11 μm . This means that larger mass of core and wall material produced much larger particle size diameter. However, Nelson [26] states in the study that microcapsules typically range in size from 1 to 20 μm .

The interaction effect of LEO and GA on the mean particle diameter at fixed 2g of CH was shown in Figure 2(b). The 3D plot was acquired by plotting the mean particle diameter (μm) on the Z-axis against two factors which are LEO (X-axis) and GA (Y-axis). The result showed that using 30g of LEO at 4g of GA was able to increase the particle diameter from about 8 μm to 10 μm . This is comparable to the finding made by Sharkawy *et al.* [19], who reported that increasing the amount of core material would result in a significant increase in the mean diameter of microcapsules.

Figure 2(c) demonstrates the interaction influence of CH and GA at a constant LEO mass of 30g. This study found the optimal value (30g of LEO) and subsequently used it for the preparation of optimum EO microcapsules, which is consistent with the authors finding [27]. From the figure, it was shown that about 2g of CH and 4g of GA showed only 8 μm to 10 μm inhibition of particle diameter. This might be due to the increase in the mass of both wall materials. Another study mentioned that mean particle diameter tends to decrease with the increase in ratio volume [21]. Therefore, it can be concluded that both factors had a simultaneous effect on particle diameter.

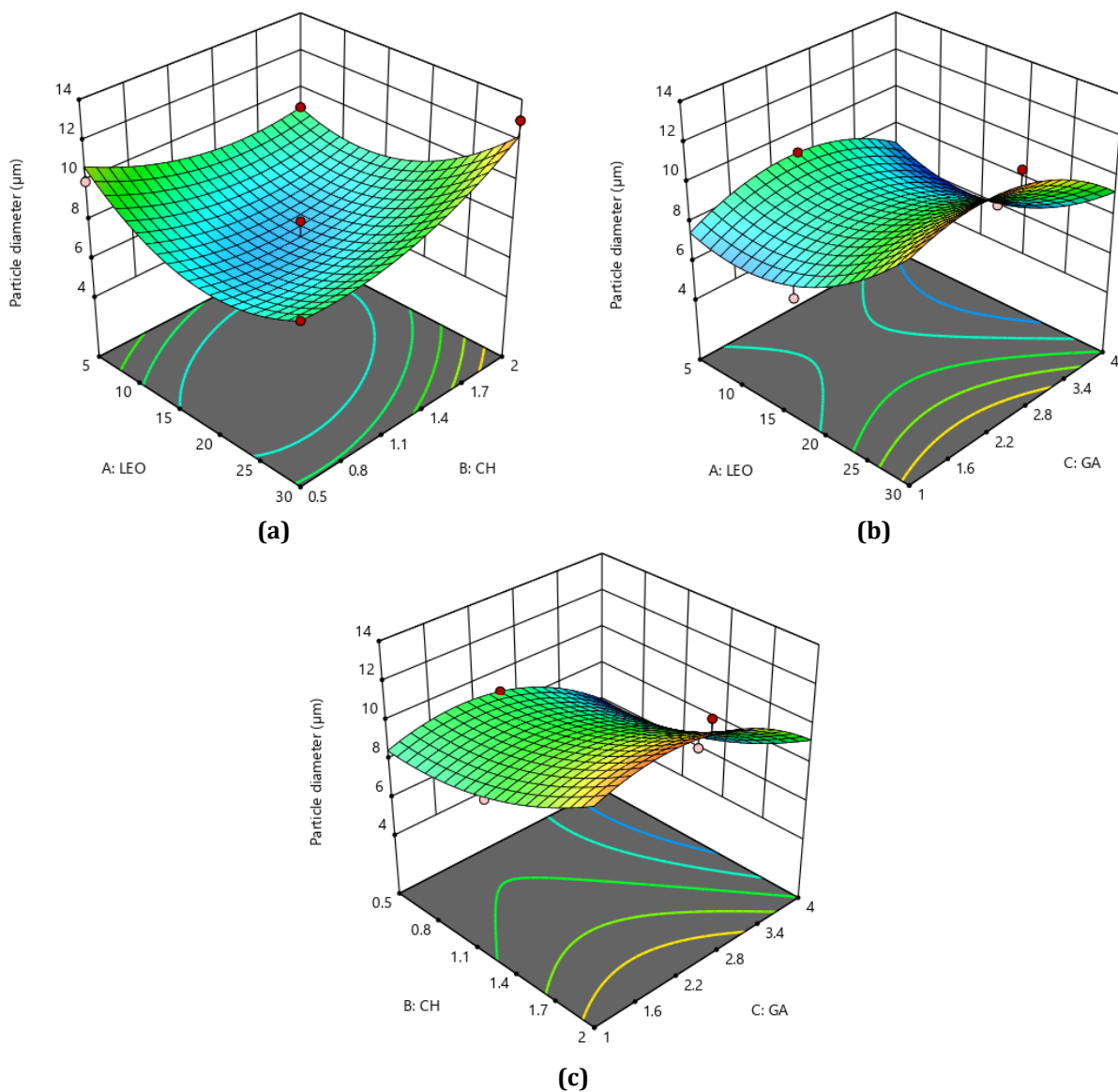


Fig. 2 Response surface plots of LEO microcapsule showing the effect of (a) LEO mass and CH mass; (b) LEO mass and GA mass; and (c) CH mass and GA mass on particle diameter

Figure 3 represents the combination effect of two material condition parameters on the encapsulation efficiency. As shown in Figure 3(a), a response surface plot for the interaction effect of LEO and CH to a fixed 2.5g of GA on encapsulation efficiency was presented. The percentage of efficiency was increased up to 85% at 30g LEO with 2g of CH. A previous study reported that GA works well for microencapsulation [28]. This is supported by another study that the encapsulation efficiency (%) of the microcapsule increased with an increase in concentration and decreased when the concentration of the therapeutic agent was higher than 4 mg/mL⁻¹ [14].

The interaction effect of LEO and GA extraction time on encapsulation efficiency at a fixed CH mass of 2.5 g was shown in Figure 3(b). The results demonstrated that the efficiency of LEO microcapsules is between 80 to 82 % of efficiency when encapsulating with 5 g of LEO and 1 g of GA. Generally, these values are much lower than the encapsulation efficiency (98.4%) of chitosan-gum Arabic microcapsules with a load of limonene [27]. According to another study, lower encapsulation efficiency could potentially be the result of some oil being lost during processing (homogenization and high-temperature spray drying), as the chemical makeup of the shell precursors is crucial to microencapsulation. Therefore, it is impossible to completely rule out chemical interactions between the active oil and the shell ingredients [29].

Figure 3(c) demonstrates the interaction effect between CH and GA on encapsulation efficiency at a constant LEO mass of 30g. From the figure, it was illustrated that the CH cause encapsulation efficiency happened at the range 82 to 85 % which can be concluded as a quite high efficiency potential. Therefore, it can be concluded that both factors are dependent on each other. Hence, the optimal predicted encapsulation efficiency of LEO microcapsules including LEO mass, CH mass, and GA mass are at 30 g, 2g, and 1g, respectively.

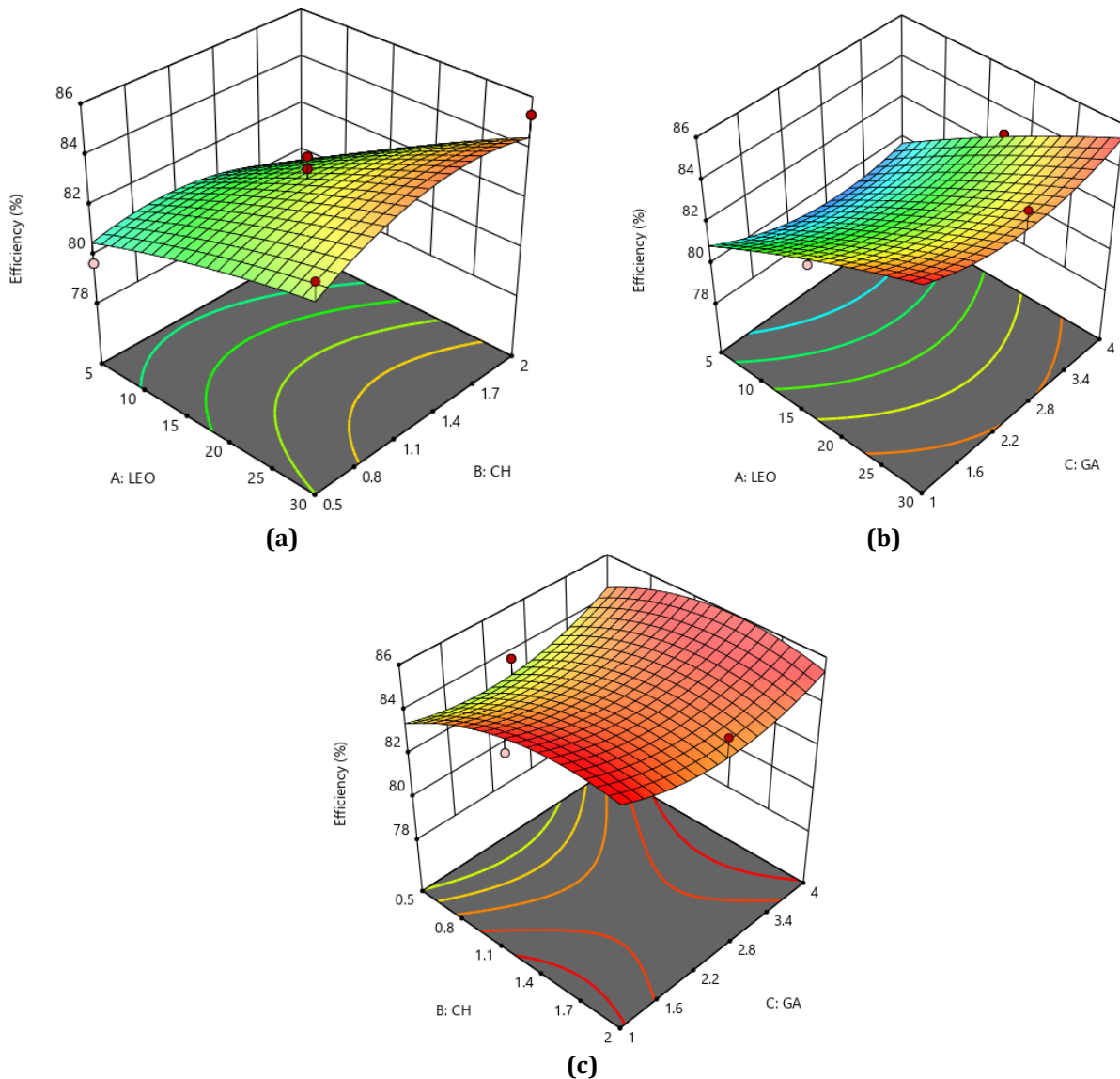


Fig. 3 Response surface plots of LEO microcapsule showing the effect of (a) LEO mass and CH mass; (b) LEO mass and GA mass; and (c) CH mass and GA mass on encapsulation efficiency

3.3 Validation of the Model

The optimal condition was identified by leveraging the desirability of the responses through statistical software [30]. The microencapsulation process was conducted under ideal conditions, and the responses were thereafter determined and verified in compliance with the prior established procedure. The ideal parameters to ascertain the particle diameter and encapsulation effectiveness of LEO microcapsules were the following: LEO mass (30g), CH mass (2g), and GA mass (1g) with desirability of 0.982. A desirability value of 0.982, close to 1, indicates that the ideal formulation performs exceptionally well across all responses. It suggests that the formulation nearly meets the ideal goals for encapsulation efficiency and particle diameter. This value was determined through the optimization process in RSM, combining individual desirability functions for each response. The high value shows that the formulation effectively balances the desired outcomes for both responses, ensuring high performance and suitability for its intended applications. The experimental results match the expected values under these ideal circumstances, with a percentage of errors less than 5%, as shown in Table 6 [30], [31]. This suggests that the model generated through Response Surface Methodology (RSM) coupled with Box-Behnken Design (BBD) was both precise and reliable in predicting the efficiency of the produced LEO microcapsules.

Table 6 Experimental data of the validation of predicted values at optimal microencapsulation conditions for LEO microcapsules

Responses	Predicted Value	Experimental Value	Percentage of error (%)
Mean particle diameter (μm)	11.76	12.00	2.04%
Encapsulation efficiency (%)	84.91	85.21 \pm 0.06	0.35%

At these optimal formulations, the experimental values obtained for the particle diameter of LEO microcapsules were 12.00 μm , which closely aligns with the predicted value of 11.76 μm . Similarly, the experimental value for encapsulation efficiency was 85.21%, which matches the predicted value of 84.91%. These findings demonstrate the efficacy of the optimized formulations. Notably, the encapsulation efficiency achieved with these LEO microcapsule formulations surpass those reported in other studies that used different combinations of polymers. For instance, Erminawati *et al.* utilized lemongrass extracts with maltodextrin and β -cyclodextrin as microencapsulates and achieved encapsulation efficiencies of only 66% and 11%, respectively [16]. This suggests that the proportions employed in the microcapsule formulations in our study represent optimal conditions for maximizing encapsulation efficiency.

Hence, the optimized formulations of LEO microcapsules demonstrate promising results, with mean particle diameter and encapsulation efficiency values closely aligned with predictions. These findings underscore the potential of the developed formulations to enhance the efficiency of microencapsulation processes, particularly when compared to previous studies utilizing different polymer combinations.

4. Conclusion

The optimization of lemongrass essential oil (LEO) microcapsule formulation conditions using Response Surface Methodology (RSM) and Box-Behnken Design (BBD) has proven to be a valuable approach for enhancing the efficiency of the studied parameters. The optimized formulation conditions were successfully verified and found to be in close agreement with the experimental values. Specifically, the best combination of LEO mass (A), CH mass (B), and GA mass (C) was determined to be 30g of LEO, 2g of CH, and 2.5g of GA, respectively. At these optimized conditions, the LEO microcapsules exhibited a particle diameter of 12 μm and an encapsulation efficiency of 85.87 \pm 0.56 %. Achieving maximum efficiency in the microcapsule formulation depends on the exact ratio of each mass component. This result emphasizes how crucial it is to precisely weigh the masses of the wall and core material to guarantee efficient and cost-effective microcapsule manufacturing.

In conclusion, the optimization of LEO microcapsule formulation conditions through RSM and BBD represents a significant advancement in the field. The optimal conditions that have been developed offer a promising avenue for upscaling production and commercialization. Additionally, they open the door for novel uses within the textile sector. Continued research efforts in this direction will further elucidate the potential benefits of LEO-based microcapsules and their impact on various industries and consumer products. Future studies should aim to further investigate the efficacy of LEO-treated fabrics, including assessments of their aromatic properties and potential functional advantages. Such research endeavors will strengthen the findings of this study and contribute to the broader understanding of LEO's utility in textile applications.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: **study conception and design:** Nur Ain Arina Johan, Siti Hana Nasir, Aisyah Mohamed Rehan; **data collection:** Nur Ain Arina Johan, Hanis Athirah Hassan; **analysis and interpretation of results:** Nur Ain Arina Johan, Siti Hana Nasir, Dzulqarnain bin Khadmudin; **draft manuscript preparation:** Nur Ain Arina Johan, Siti Hana Nasir, Mohamad Faizul Yahya. All authors reviewed the results and approved the final version of the manuscript.

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